REMARKS

Claim 1 has been amended without any intention of disclaiming equivalents thereof. New claim 48 has been added. Upon entry of this paper, claims 1-4, 6-8, 13, 19-21, 23, 24, 26-28, 30-34, 42, and 44-48 will be pending and under consideration.

Claim 1, step (1) has been amended to recite that *at least one PET is* unambiguously indicative of the presence in the sample of the alternative splicing form protein from which it is derived and comprises an amino acid sequence encoded by an RNA spanning a splice junction. Claim 1, step (2) as currently amended recites, in part, that at least one of the plurality of capture agents specific for respective PETs is *specific for the at least one PET comprising an amino acid sequence encoded by an RNA spanning a splice junction*. New claim 48 reflects the subject matter of claim 1, but recites that either of the first or second capture agents respectively specific for the PET and a site separate from the PET on a polypeptide analyte is immobilized on a solid support and the other capture agent is labeled with a detectable moiety. Support for the amendments to claim 1 and new claim 48 appears throughout the application as filed, for example, on page 98, lines 27-28, and from page 42, line 24 to page 43, line 7 of the application as filed.

Applicants believe that the aforementioned amendments and new claim introduce no new matter.

Rejections Under 35 U.S.C. § 103(a)

According to Section 4 of the outstanding Office action, claims 1, 3-4, 19, 24, 26, 28, 30-34, 42, 44, and 46 have been rejected under 35 U.S.C. § 103(a) as being obvious in view of a combination of four different references including Dours-Zimmermann *et al.* (1994) J. Biol. Chem. 269(52): 32992-98 ("Dours-Zimmermann"), Hongo *et al.* (1994) Journal of General Virology 75: 3503-3510 ("Hongo"), Jemmerson *et al.* (1987) Proc. Natl. Acad. Sci. USA 84: 9180-84 ("Jemmerson"), and Arenkov *et al.* (2000) Anal. Biochem. 278: 123-31 ("Arenkov"). According to Section 5 of the outstanding Office action, claims 2, 6-8, 13, 20, 21, 23, 26, 27, 45, and 47 have been rejected as being obvious over a combination of five different references

including Dours-Zimmermann, Hongo, Jemmerson, Arenkov, and United States Patent No. 6,897,073 by Wagner *et al.* ("Wagner").

In the previous Office action, dated August 10, 2007, similar rejections under 35 U.S.C. § 103(a) were raised, based on the combination of Dours-Zimmermann, Jemmerson, and Arenkov, and on the combination of Dours-Zimmermann, Jemmerson, Arenkov, and Wagner. According to the present Office action, those previous rejections under 35 U.S.C. § 103(a) have been withdrawn because Dours-Zimmermann, Jemmerson, Arenkov, and Wagner "do not teach the limitation of RNA spanning a splice junction." (Office action, page 9, Section 6.)

In the present Office action, Hongo has been added to each combination of references that was cited previously. The present Office action alleges that "Hongo et al. discloses synthesizing unspliced and spliced mRNAs from the same segment of RNA that contains a splice junction and translating those mRNAs to yield proteins (abstract; page 3503, left column). Furthermore, Hongo et al. teach detecting polypeptides encoded by mRNA using a molecular assay (abstract)." (Office action, page 4, third full paragraph.) While not necessarily agreeing with these statements, Applicants respectfully submit that Hongo, alone or in combination with the other cited references, fails to teach or suggest using a capture agent *specific for a PET or recognition sequence that includes an amino acid sequence encoded by an RNA spanning a splice junction*, as required by each of Applicants' independent claims.

Specifically, the claimed subject matter of the present invention enables the measurement of proteins in a sample that include one or more proteins that are expression products of an alternative splicing form of DNA. Sample proteins are fragmented using a predetermined protocol to generate peptide epitope tags (PETs) that are unambiguously indicative of the target proteins in the sample. Then, an immobilized capture agent and a labeled capture agent are used to bind the PET and another site on the fragments that include the PETs and detect the presence, amount, and/or absence of the respective target proteins. Applicants have amended independent claim 1 of the present invention to recite, in part, that at least one of the capture agents is *specific for the at least one PET comprising an amino acid sequence encoded by an RNA spanning a splice junction*. Independent claims 30, 42, and 48 include similar subject matter.

Hongo describes the identification of an unspliced form of mRNA coming from an RNA segment (segment 6) that codes for Influenza matrix protein (M1). Specifically, Hongo isolated RNA segment 6 specific mRNA from virus-infected cells and showed that the unspliced mRNA form is present, but at lower quantities than the spliced form that codes for the protein M1. (Abstract.) Based on the sequence of the unspliced mRNA, Hongo deduced that translation of the unspliced form would add an extra 132 amino acids (denoted CM2 and shown as deduced residues 243 to 374 in Figure 2) to the C-terminus of the M1 protein. The amino acid sequence of the M1 protein is shown as residues 1 to 242 in Figure 2. The primary splice junction described in Hongo corresponds to the location between residues *242 and 243* in Figure 2, which respectively correspond to the C-terminus lysine of M1 and the first deduced amino acid (tryptophan) of CM2. This splice junction is identified in Figure 1 at nucleotide 754, which appears in the deduced tryptophan codon (TGG).

To determine if the unspliced form of mRNA was in fact translated to protein, Hongo generated antiserum against a 122 amino acid segment of CM2 that corresponds to *residues 253 to 374* in Figure 2 (in a GST fusion protein). (Page 3505, right column.) Hongo immunoprecipitated proteins in cell lysates from cells infected with the Influenza virus using the antiserum to the CM2 segment and anti-M1 monoclonal antibodies. With the antiserum generated against the CM2 segment, Hongo immunoprecipitated a polypeptide with an apparent $M_r \approx$ 18K, which Hongo describes as CM2, along with a heterogenous mixture of polypeptides with apparent $M_r \sim$ 22K to 30K. With various anti-M1 monoclonal antibodies, Hongo immunoprecipitated no polypeptides other than the M1 polypeptide, which has a C-terminus at residue 232 in Figure 2. (Page 3509, left column.)

It is important to note that Hongo's antiserum is generated against residues 253 to 374, which according to Figure 2 begins ten residues downstream from the M1 C-terminal lysine (at residue 242) that corresponds to the splice junction at nucleotide 754 in Figure 1. Accordingly, there is no indication in Hongo that either the antiserum against CM2 or the antibodies against M1 are specific for any amino acid sequence that is encoded by an RNA splice junction. For example, neither the antiserum against CM2 nor the antibodies against M1 appear to be specific for an amino acid sequence that is encoded by the splice junction at nucleotide 754 in Figure 1.

Clearly, like Dours-Zimmermann, Jemmerson, Arenkov, and/or Wagner, Hongo is not concerned with, and does not contemplate, using a capture agent *specific for a PET or recognition sequence that includes an amino acid sequence encoded by an RNA spanning a splice junction*, as required by each of Applicants' independent claims. Accordingly, because Dours-Zimmermann, Jemmerson, Arenkov, Wagner, and/or Hongo, alone or in combination, fail to teach or suggest at least this element of Applicants' independent claims, Applicants respectfully submit reconsideration and withdrawal of these rejections.

CONCLUSION

Applicants invite the Examiner to contact the undersigned Attorney to discuss any remaining issues with this application. Applicants believe that the claims are in condition for allowance. Early favorable action is respectfully solicited.

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Respectfully submitted,

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